

Histological investigations on *Dendrodoris nigra* (Stimpson, 1855) (Gastropoda, Nudibranchia, Dendrodorididae)

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Abstract

The histology of the major organ systems (digestive, reproductive, nervous, circulatory, excretory and respiratory, as well as epidermis) of the nudibranch *Dendrodoris nigra* (Stimpson, 1855) are described for the first time and the results are compared with those derived from other members of the Doridoidea. It is shown that some characters which have been used to differentiate the genus *Dendrodoris* Ehrenberg, 1831 from other doridoideans (i.e., retractability of gills, lack of hard structures in the anterior digestive system, presence of pericardial glands) are problematic when used for phylogenetic analysis. This is especially true when taking into consideration that little is known about details of these structures in the Doridoidea as a whole.

Key words: Nudibranchia, Doridoidea, *Dendrodoris nigra*, histology, phylogeny

Introduction

Two recent papers dealing with the taxonomy of species belonging to the genus *Dendrodoris* Ehrenberg, 1831 have clarified the taxonomy of species from the Indo-Pacific (Brodie et al. 1997) and the Atlantic (Valdés et al. 1996), and demonstrated complexes of highly variable species in both instances. Although the external features may be very variable in some species e.g. *Dendrodoris nigra* (Stimpson, 1855) and *Dendrodoris grandiflora* (Rapp, 1827), the internal organ systems seem to be rather more conservative with relatively little variation (Brodie et al. 1997). Furthermore, a comparison of the organ systems of different *Dendrodoris* species shows that *Dendrodoris* is a well marked genus within the family Dendrodorididae O'Donoghue, 1924. The synonymy and validity of genera within the Dendrodorididae have been reviewed recently (Valdés et al. 1996; Valdés & Ortea 1997).

The family Dendrodorididae is unusual within the Doridoidea in that it lacks cuticular structures within the buccal mass. This character is shared by the Phyllidiidae Rafinesque, 1815, a family with secondary gills on the ventral side between the notum and the foot. The absence of jaws and radula is the single character which, according to Bergh (1876), unites these two families, and for which he created the taxon Porostomata. Although many authors have followed his classification (Pruvot-Fol 1954, Odhner in Franc 1968, Schmekel & Portmann 1982, Thollessen 1998), some mentioned doubts and considered the taxon an artificial grouping (Brunckhorst 1993, Rudman 1998). Since only the lack of characters unites these two rather different families, there is a demand for further characters to support or to falsify the relationship. Brunckhorst (1993: 84)

came to the conclusion, after analysing the phylogenetic relationship within the Phyllidiidae, using *Dendrodoris* and *Chromodoris* as outgroups, that *Dendrodoris* shared more derived characters with the Cryptobranchia than with the Phyllidiidae. He concluded "the grouping of dendrodorids and phyllidiids together as the Porostomata is untenable.... The Dendrodorididae clearly belong with other doridoids ... but their phylogenetic position is yet to be clarified." This view was diametrically opposed by Valdés (1996) who stated that "the superfamily Porodoridoidea is a monophyletic group" and that "the loss of the radula has only occurred once in the evolution of the dorids." There is no doubt that this situation requires further clarification.

In this paper we present characters of *Dendrodoris nigra* which are difficult to find by macroscopic investigation and which will help to elucidate the phylogenetic relationship of the Dendrodorididae. Although we present several characters in a new light, it is evident that only after investigating many more members of the Doridoidea with the same thoroughness that the relationship of the Dendrodorididae can be determined.

Material and Methods

Three specimens of *Dendrodoris nigra* (length of living specimens 23, 43, 53 mm) from Dingo Beach (Queensland, Australia, collected by the authors in August 1995 and July 1997) were embedded in hydroxyethylmethacrylate for serial sectioning (2-3 μ m). Sections were stained with toluidine blue. Comparisons were made with sections of other doridoideans belonging to the Cryptobranchia and Phanerobranchia. For anatomical drawings and general description of the organ systems of *D. nigra* the reader is referred to Brodie *et al.* (1997).

Results

Epithelia

Epidermis: The dorsal notal epithelium consists mainly of high columnar to flask-shaped cells with basally lying nuclei (Fig.1A). The cytoplasm stains homogeneously light blue to transparent without further differentiation. Cells of similar size with several smaller, light blue staining grana and cells with a dark violet staining network (cells producing acid mucopolysaccharids) are interspersed. Very few ciliated cells can be observed. Black pigment grana, which obviously are not confined to a certain cell type, are located in the connective tissue of the notum beneath the basal lamina of the epidermis. The epithelium on the ventral side of the notum shows similar cell types, but the cells are less tall. In the dorsal area, the epithelium very often forms grooves or invaginations, which do not differ in their cellular appearance from the rest of the epithelium (Fig. 1B). Subepithelial glandular follicles composed of one or several cells are present in the lateral part of the notum. These glandular cells are similar in appearance to the acid mucopolysaccharide producing cells in the epidermis, but staining slightly darker.

Rhinophores: The epithelium consists of small, prismatic cells with basally lying nucleus and no visible differentiation of cytoplasm. Some glandular cells are interspersed, containing a large light blue staining vacuole. The margins of the

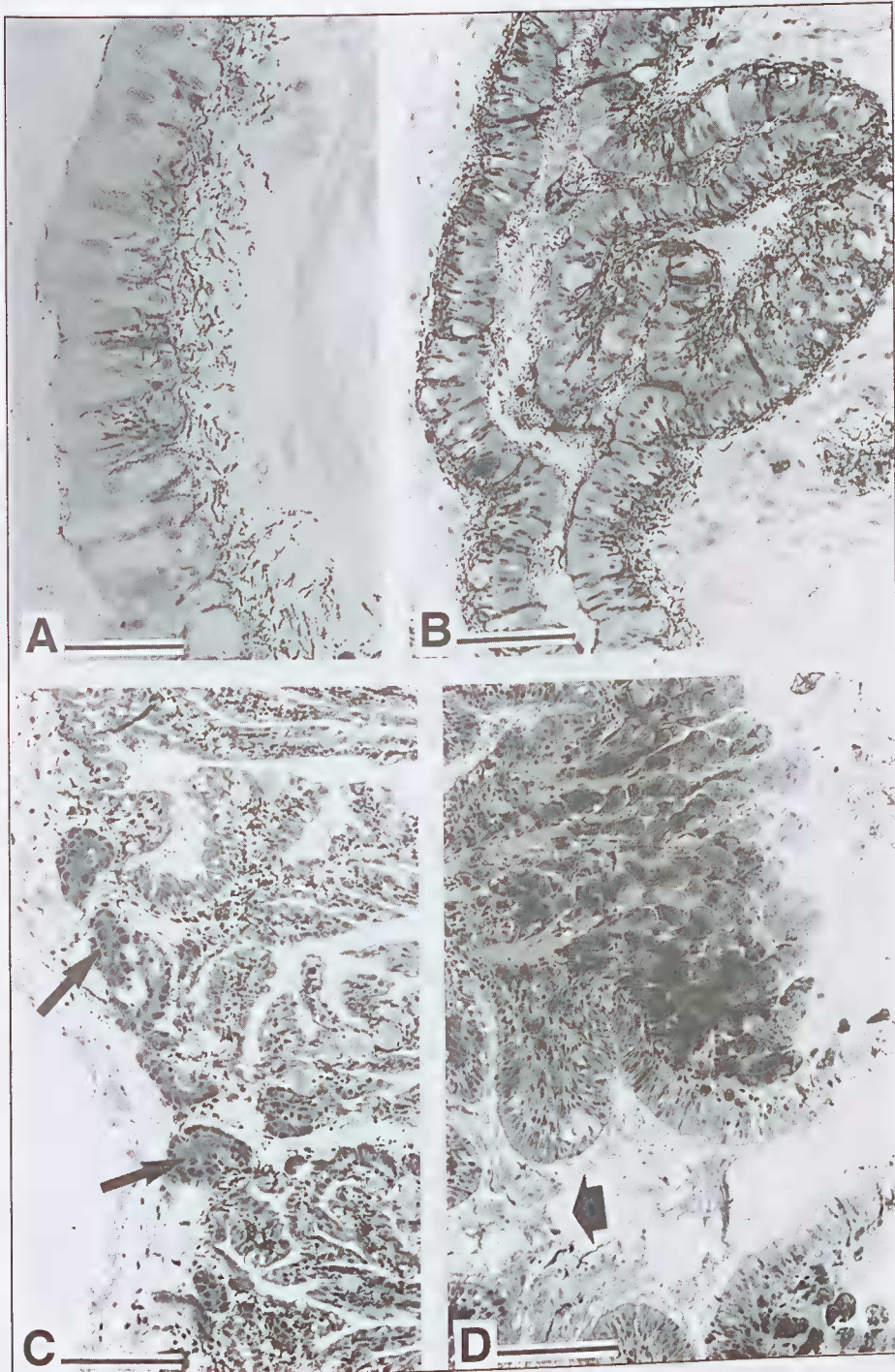


Figure 1. *Dendrodoris nigra*. Epithelia. **A**, dorsal notum epithelium with underlying black pigment granules; **B**, dorsal notum epithelium of an invagination; **C**, basal part of gills with gill glands (arrows); **D**, glandular follicles around the mouth; arrow indicates the direction to the mouth. Scale bars: A 50 μm , B-D 100 μm .

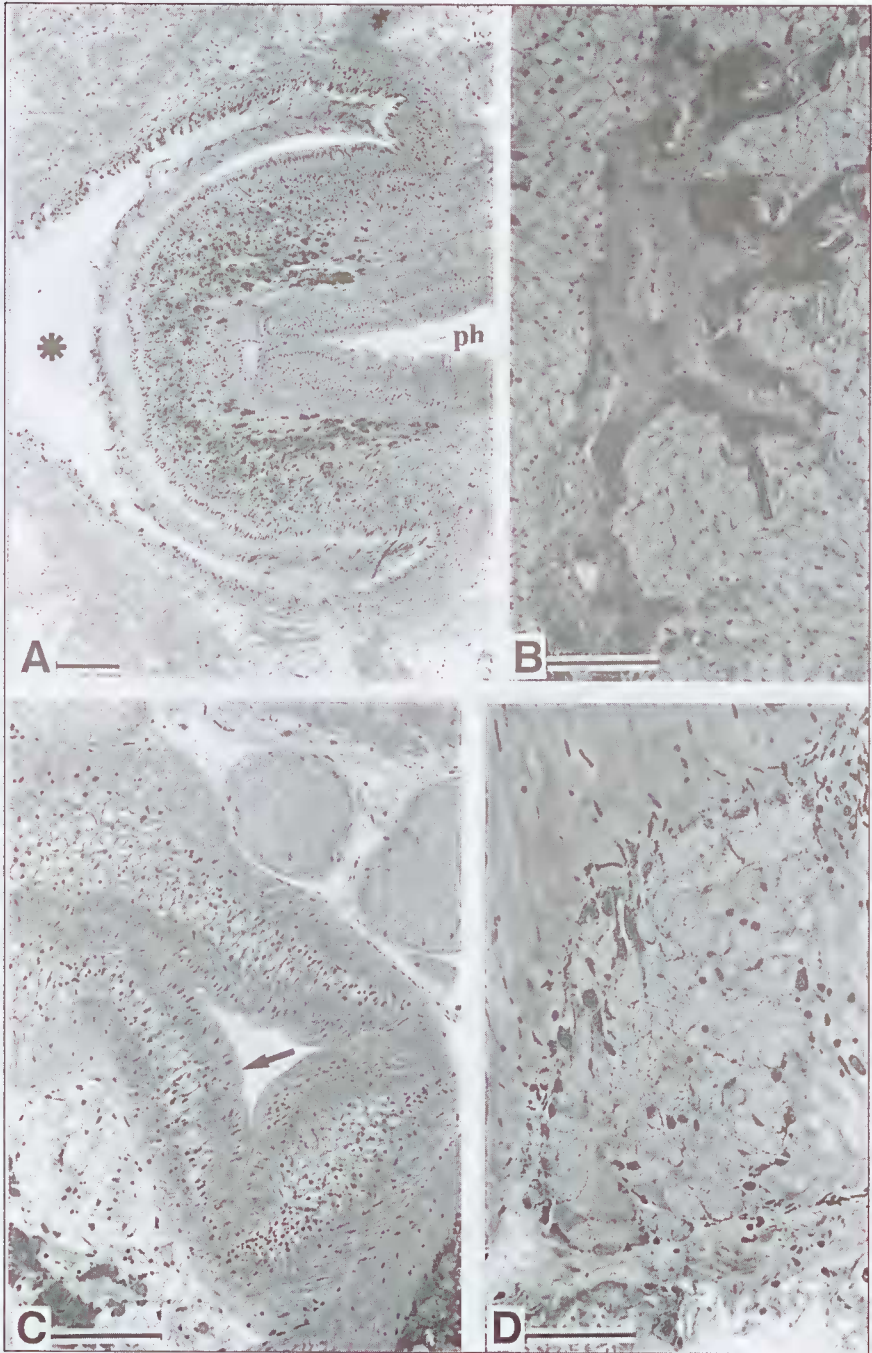


Figure 2.

Dendrodoris nigra. Digestive tract. **A**, transverse section of labial disc with pharynx on the right and oral tube (asterisk) on the left side; **B**, oral gland with cross section of collecting ducts, annexed glandular area staining dark (arrow) and glandular area staining light; **C**, semi cross section of pharynx; cuticular lining (arrow) barely visible; **D**, cross section of right salivary gland. Abbreviations: **ph** - pharynx; Scale bars: A-C 100 μm , D 50 μm .

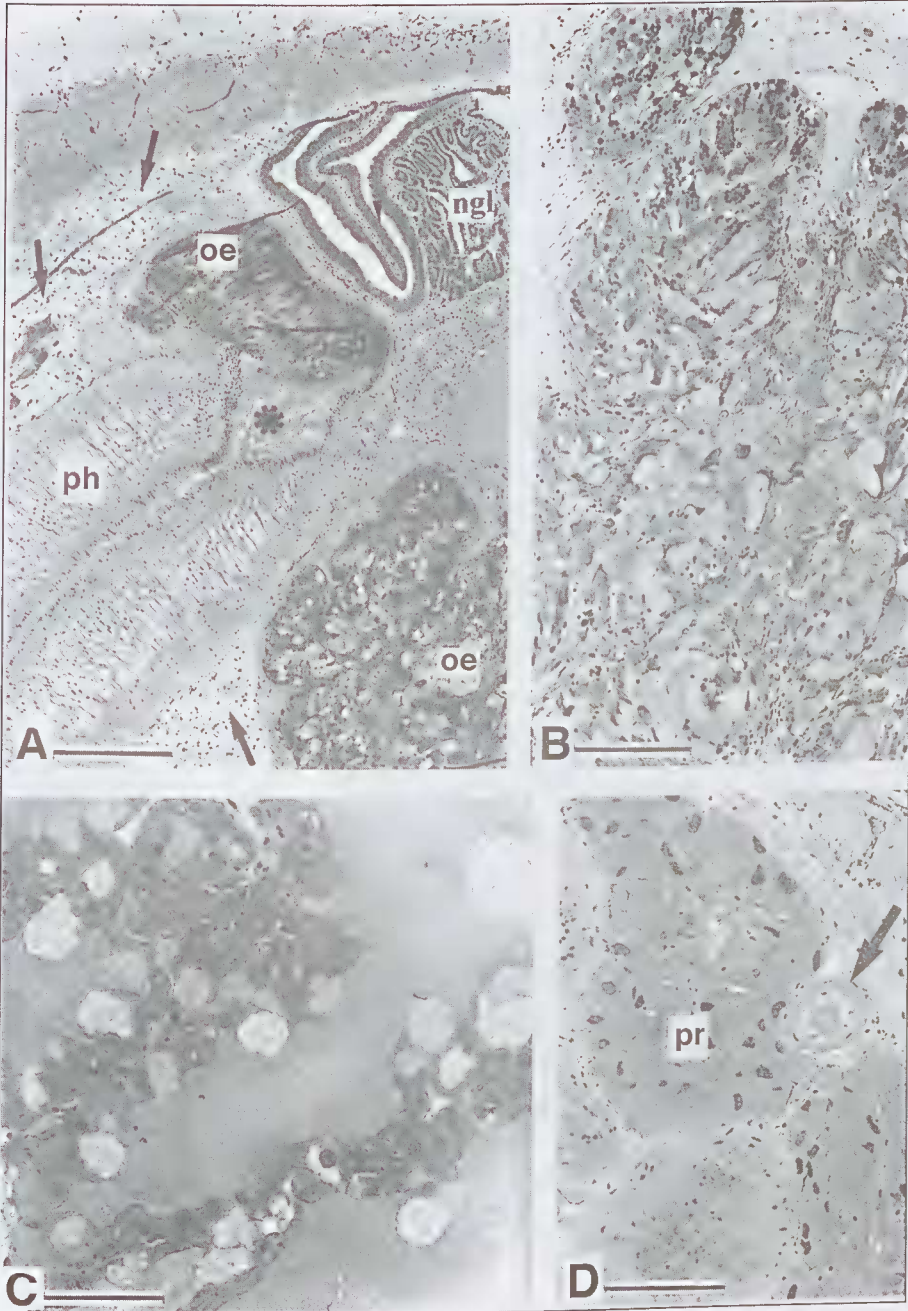


Figure 3.

Dendrodoris nigra. A, cross section showing transition from pharynx into oesophagus (asterisk) with proximal part of oesophagus above the asterisk and on bottom on right side, juvenile nidamental glands and oral gland on the left side above pharynx (arrows); B, part of distal oesophagus; C, cross section of digestive glandular lobes, D, cross section of vas deferens (arrow) and prostatic duct with subepithelial glandular follicles of prostate gland. Abbreviations: ngl - nidamental glands, oe - oesophagus, ph - pharynx, pr - prostate gland; Scale bars: A-D 100 μm.

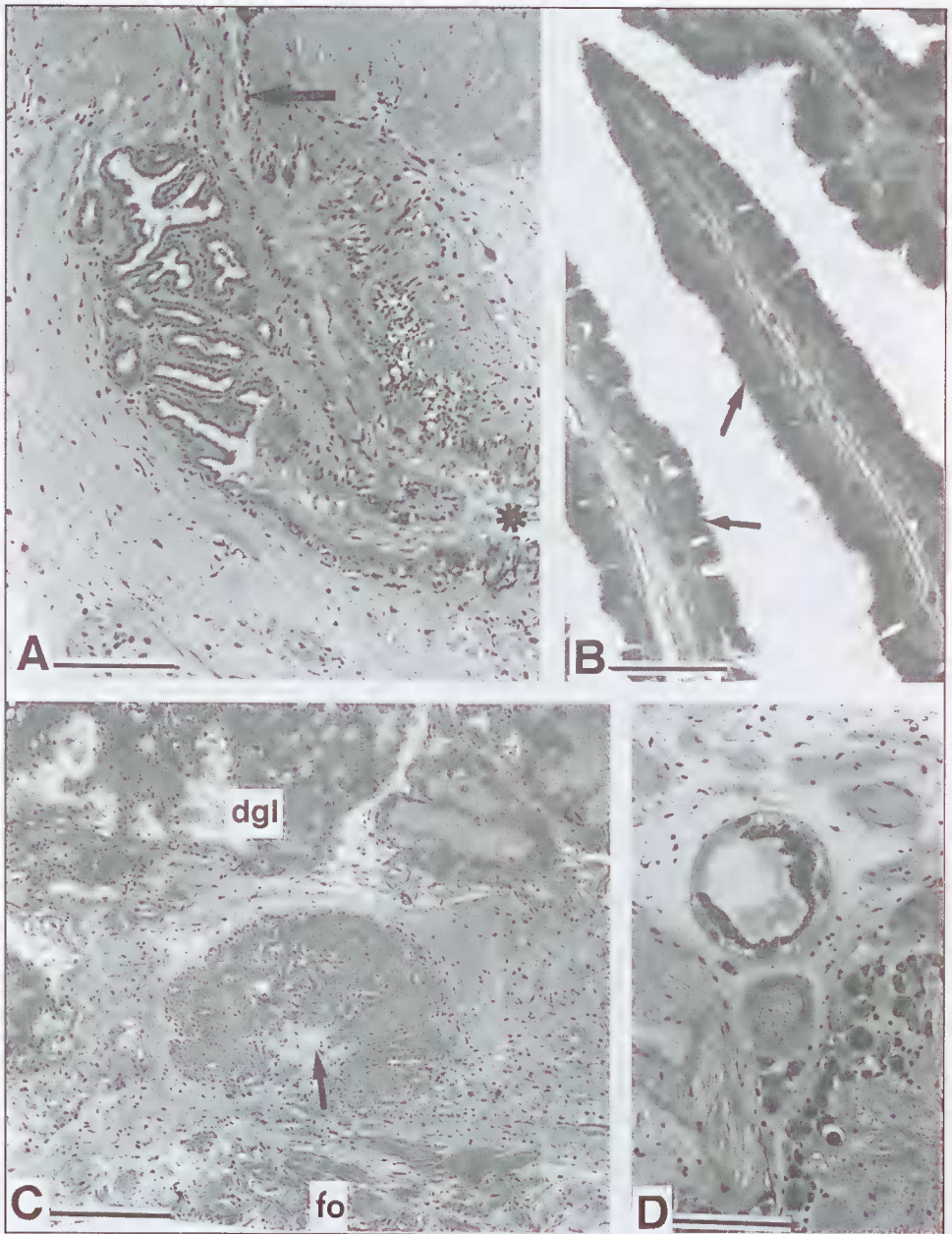


Figure 4.

Dendrodoris nigra. **A**, vestibular gland of immature specimen; asterisk indicates the opening into the vestibulum; arrow indicates the distal oviduct; **B**, detail of vestibular gland of mature specimen; epithelium showing a high microvilli border (arrows); **C**, blood vessel situated between the fused retractor muscles (arrow); **D**, eye with optic ganglion beneath and cerebro-pleural complex on right side. Abbreviations: fo - foot, dgl - digestive gland intermingled with excretory system. Scale bars: A, C-D 100 μm , B 50 μm .

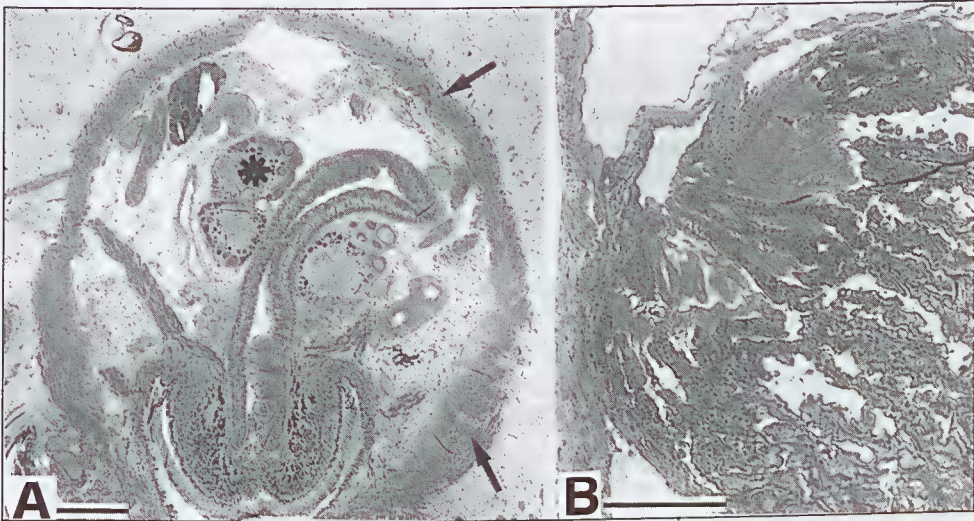


Figure 5.

Dendrodoris nigra. **A**, cross section of anterior part of body, showing body cavity framed by a thick muscle layer (arrows), labial disc with pharynx, pedal ganglia on both sides of the pharynx and the pleural (asterisk) only on the left side; **B**, cross section of syrinx. Scale bars: A-B 100 μm .

rhinophoral lamellae of one animal shows few larger cells containing non-staining vacuoles, similar to the special vacuole cells of other Doridoidea (see Wägele 1998).

Foot epithelium: The cells are tall and ciliated, with nuclei lying basally to medially. Few epithelial glandular cells with violet staining grana are present. Subepithelial glandular follicles with two to four glandular cells containing blue to violet grana are very common.

Gill (Fig. 1C): The epithelium consists of cuboidal cells with large nuclei. Glandular cells, which stain homogeneously light blue or have violet grana, are interspersed. Ciliated cells are also present. Gill glands (Fig. 1C, arrows) are present at the bases of the gills. They are composed of many cells with bluish contents and large nuclei. The gills are underlaid by thick muscle layers, concentrating anteromedian first in one U-shaped muscle, later separating into two strings running ventrally to the head region.

Digestive tract

Oral tube: The epithelium consists of high columnar cells interspersed with some glandular cells with bluish contents and a few glandular cells containing violet grana. A thick glandular layer, composed of pyriform glandular follicles filled with grana of rather uniform size, staining bluish violet, surrounds the mouth area (Fig. 1D).

Labial disc (Fig. 2A): The labial disc is not covered by a cuticle. Scattered glandular cells containing vacuoles uniformly staining violet can be observed. Two strong muscles insert at the labial disc (Fig. 5A).

Oral (ptyaline) gland (Fig. 2B): the oral gland is formed by a thick layer of glandular cells resulting in a spongy appearance. The large vacuoles in these cells, which have pycnotic nuclei, do not stain. Along the outgoing ducts glandular cells with bluish contents can be observed. The two main ducts are characterized by a thick ring muscle layers. After fusion of these ducts, the common duct leads ventrally along the pharynx within the labial disc and opens into the lumen of the oral tube.

Pharynx (Fig. 2C, 3A, 5A): The pharynx is tube-like and long and is composed of several layers. From outer to inner side following layers can be observed: exterior circular muscle layer with areas of transverse muscles; layer of cells with large vacuoles giving the appearance of a network; layer of circular muscles; pharyngeal epithelium without any glandular cells; thin cuticle.

Salivary glands (Fig. 2D): The salivary glands form a rather compact circle around the transition between pharynx and oesophagus. Only one microscopic duct leading into the pharynx is observable. No distinct lumen within the glandular tissues is visible, and no distinct arrangement of glandular cells into an epithelium is discernible. The glandular cells exhibit a medium sized functional nucleus, less commonly a pycnotic nucleus. They are completely filled with densely packed, very light blue staining grana, showing a superficial similarity to the oral glandular tissue.

Oesophagus (Fig. 3A, B): The oesophagus is surrounded by a layer of circular and longitudinal muscle fibres of varying thickness. No lumen is observable throughout the oesophagus except for the most anterior part, which is filled with pink secretions. It is difficult to describe the number of different glandular cell types, since all different types may represent different functional phases of one and the same type. Following phases are discernible: cells with blue to violet staining large vacuoles; cells with reddish to violet staining large vacuoles; cells with different coloured (from pink to light violet) but rather homogeneously staining cytoplasm. The nuclei always lie basally.

A further glandular cell type with filiform contents, staining dark violet, is present in the anterior part of the oesophagus (Fig. 3A). In the distal part of the oesophagus, the cells with homogeneously staining contents dominate (Fig. 3B). The whole oesophagus is underlaid by an extremely thick muscular layer.

Stomach: Especially at the entrances into the digestive gland, the stomach wall is folded. The epithelium is composed of small, elongate and ciliated cells with small, elongate, basally lying nuclei.

Digestive gland: Many entrances from the stomach area into the digestive gland are visible. Probably only one type of glandular cells is present in the digestive gland, since transitional stages can be observed: early stage cells contain small bluish grana or vacuoles, increasing in size in the following phases and forming a grape-shaped (clustered) structure, now staining more greenish to light blue (Fig. 3C). When contents of vacuoles is emptied, small spaces or areas with microscopic grana staining violet remain.

Caecum: No caecum (transformed right digestive gland) opening into the stomach is distinguishable.

Intestine: The inner wall of the intestine is folded throughout its length. After its origin from the stomach the intestine is especially highly convoluted. No typhlosole is discernible. The epithelium consists of elongate, non-glandular cells, with long cilia and basally to medially lying nuclei. Some violet staining glandular cells are interspersed in the proximal part of the intestine that follows after bending and running posteriorly. The intestine is surrounded by a layer of circular muscles followed (inwards) by a ring of longitudinal muscles. No specialization of cells is discernible in the area where the intestine bends and then runs posteriorly, called caecum or pyloric gland by different authors respectively (Brodie *et al.* 1997; Valdäs *et al.* 1996).

Reproductive system

Gonad and ampulla: The gonad forms an incomplete layer over the digestive gland, with parts extending deep into the digestive glandular tissue. In cross section the digestive glandular tissue appears intermingled with gonad and kidney. The ampulla is sausage-shaped and filled with autosperm. The epithelium of the ampulla is composed of cuboidal to flat cells.

Distal genital tract: The vas deferens shows a thick tubular prostate gland. The glandular cells lining the duct are elongate, filled with light blue staining grana and basal nuclei. A dense ciliation is present within duct. Subepithelial glandular cells are enormous, of similar appearance as the epithelial glandular cells, but with large nuclei, arranged into follicles (Fig. 3D). The proximal and distal sections of the vas deferens (non-prostatic parts) are lined by an epithelium with cuboidal, ciliated cells only. A layer of circular muscles surrounds the non-glandular parts. No cuticularized penial structures (i.e. cuticular lining and/or spines) are visible in any of the specimens investigated.

In the smaller specimen the female mass is not differentiated into distinct glands (Fig. 3A), but in the two larger ones it is composed of three distinct parts, namely the capsule gland, proximal and distal mucous glands. The capsule gland consists of numerous narrow coils. The cells of the epithelium are broad and columnar containing numerous, large, light-blue staining granules.

Spherical to elliptic nuclei are lying at the base of the glandular cells. Supporting cells with short cilia are alternating with the glandular cells. The proximal mucous gland is connected to the capsule gland by a long coiled duct lined by densely ciliated cells. The epithelium of the proximal mucous gland is folded in narrow coils similar as in the capsule gland. The glandular cells are prismatic and contain heterogeneous mucous secretions staining purple or dark red. Supporting cells bear very long cilia. The distal mucous gland is composed of various parts with different staining properties. These may represent various functional stages and are therefore difficult to interpret. All glandular cells of the distal mucous gland contain purple to red staining mucous secretions. The basally lying nuclei of the glandular cells are pycnotic. The supporting cells have long cilia.

The opening of the oviduct lies more caudally than the openings of the vagina and the vas deferens. The vaginal duct is narrow and opens proximally into the vestibulum next to the vas deferens. The epithelium of the bursa copulatrix is composed of apocrin secreting cells. Only a few muscle cells surround the bursa. The receptaculum seminis is filled with allosperm, these being orientated but not attached to the folded wall. Contrary to the bursa, a distinct layer of muscles surrounds the receptaculum.

In the smaller specimen, the vestibular gland lies completely within the notal tissue and therefore is not visible anatomically in this animal (Fig. 4A). In the larger specimens, it intrudes into the visceral cavity as a compact mass. The cells are small, elongate, with basally (in smaller) to medially lying nuclei (in larger specimens) and apically lying, microscopic, violet-staining grana. The cells in the larger specimen exhibit a brush-like appearance (Fig. 4B).

Nervous system

The circumoesophageal central nervous system, with fused cerebral and pleural ganglia, surrounds the pharynx (Fig. 5A). All ganglia are surrounded by a thin layer of connective tissue. Nerve cells are concentrated in the periphery of the ganglia. The statocyst has many elongate otoconia (length about 5 μm). The eye is connected to a small optic ganglion by a very short optic nerve (Fig. 4D).

Circulatory system

In the anterior dorsal wall of the pericardium folds ("pericardial glands") are present, which are not glandular, but are composed of tiny cells and small nuclei being similar in appearance to those of the connective tissue.

A single, well formed blood vessel is present, which runs anteriorly in the ventral side of the muscle layer surrounding the visceral cavity, and posteriorly within the two fused gill retractors (Fig. 4C); sending ducts into the foot in two directions.

The blood gland is characterized by minute cells and nuclei, forming a rather compact mass on the right side of body next to the cerebropleural complex, with lacunae interspersed.

Excretory system

The bulb-like syrinx is extremely large, being approximately equal in size to the gill chamber in cross section. Only in the apical part longitudinal folds with cilia are present, whereas the basal section exhibits folded lamellae (Fig. 5B). The tissue of the kidney is not only surrounding the whole digestive gland, but also intruding into it and intermingled with the gonad in many areas.

Black pigment

The black pigment is not confined to a certain cell type. Pigment granules are widely distributed within the connective tissue and around the viscera, beneath external epithelia (including rhinophores and gills), in the peritoneal wall, between subepithelial glandular follicles around the mouth opening, within the blood gland and in the thick muscle layer surrounding the visceral cavity.

Rhogocytes

Cells with a spindle-shaped appearance filled with dark stained granules of different size and a medium sized nucleus are present throughout the notal tissue.

Discussion

Dendrodoris nigra differs not only in its anatomy from other Doridoidea (Valdés *et al.* 1996, Brodie *et al.* 1997) but also in its histology. Contrary to many other doridoideans, the epithelium of the notum consists mainly of a glandular cell type which, according to its bluish staining, does not produce acid mucopolysaccharides. Glandular cells that do produce acid mucopolysaccharides (violet staining) are interspersed with those non-glandular cells. Avila and Durfort (1996) investigated the lateral notal epithelium of *D. limbata* by electron microscopy. They also described two different types of secreting cells. The type they called mucous cell is probably the same one that is identified as secreting acid mucopolysaccharides here. They called the second type "macrovacuolated cells" and assumed that these cells are responsible for the synthesis, storage and secretion of defensive substances. We assume that this is the dominant cell type described here and which is filled by light blue contents. We can confirm the results of Avila and Durfort (1996) on the concentration of subepithelially lying glands in the lateral part of the notum producing acid mucopolysaccharids; these are less conspicuous in the smaller animal.

No special vacuolated cells (vacuolated cells Wägele 1998 = "Spezial-vakuolenzellen" Schmekel 1982) were present in either the mantle or the rhinophoral epithelium where they can usually be found. Presence of special vacuolated cells is a character that unites all members of the Nudibranchia (Schmekel 1982, Wägele 1997 & 1998). Avila and Durfort (1996) did not mention any special vacuolated cells in their descriptions on the epithelia of the notum and rhinophores of *Dendrodoris limbata*. The presence of somewhat higher vacuolated cells on the margins of the rhinophoral lamellae in one of our specimens indicate that special vacuolated cells may be present, but this has to be confirmed by ultrastructural investigations. If these cells really are those described by

Schmekel (1982) and Wägele (1998), then they differ in their size from the typical special vacuolated epithelium.

Gill glands are present in many Cryptobranchia (*Jorunna*, *Hypselodoris*) and Phanerobranchia (*Corambe*, *Onchidoris*, *Roboastra*). The absence is confirmed only for *Limacia clavigera* (Wägele 1998) amongst those nudibranchs investigated to date. Wägele (1997) considered the presence of gill glands as a synapomorphy of the Doridoidea. The genus *Dendrodoris* shares this character with all other doridoideans and is therefore indisputedly part of this clade.

Dendrodoris species can retract their gills completely. At least in *D. nigra* investigated here, the muscles responsible for this retraction are grouped first into several, then into just one, U-shaped retractor which runs along the ventral side before it separates again into two parallel stripes running ventrally in the area of the head. Up to now, this is the only species where muscles of this type and magnitude have been described (unpublished results of senior author). *Bathydoris* has no muscles in the gills or the gill base, whereas some Phanerobranchia do have muscles within the gills or gill base (*Limacia*, *Roboastra*). In *Onchidoris*, a member of the Phanerobranchia with nearly contractile gills, two larger retractors exist. The cryptobranchs *Jorunna*, *Hypselodoris* and *Discodoris* also have two main retractors, which run anteriorly on the ventrolateral sides within the musculature surrounding the visceral cavity. Although the retraction of gills into a cavity is considered to be an autapomorphy of the Cryptobranchia, we find similar features in phanerobranchs, as well as in *Dendrodoris*. The presence of just one retractor muscle in the posterior part, which runs immediately to the ventral side in *D. nigra* is a quite different situation compared to the two lateral muscles in other Cryptobranchia. Two different evolutionary trends can be discussed. First it can be assumed that the two retractors in *D. nigra* represent the two separated retractors of members of the Cryptobranchia, but in the former they migrated to the ventral side and fused at least partly. Secondly one can interpret them as homologous structures that evolved in parallel. Then they evolved from primarily homologous muscles present in nearly all Doridoidea to form retractors that do not have the same position as the retractors of the Cryptobranchia or Onchidorididae, but having the same function.

Paired oral glands are present in several groups of Nudibranchia, but only a few have a common duct opening on the ventral side into the oral tube, e.g. *Armina* (Kolb 1998), *Hancockia* (Hoffmann 1939), *Calma*, *Spurilla*, *Berghia*, *Facelina* (Dreyer 1913). According to observations on the histology of oral glands in general (unpublished results of senior author), the spongy appearance of the glandular tissue in *Dendrodoris* seems to be unique and therefore a homology with other oral glands cannot be taken for granted. The evolution of a paired oral gland with one efferent duct in the Dendrodorididae is considered to have occurred independently from other nudibranchs with oral glands.

The very large labial disc that is not covered by a labial cuticle marks the transition from the oral tube into the pharynx. Evidence for homology of the elongate, tubular pharynx in *Dendrodoris* and the (usually) short, bulbous

pharynx of other dorids is given both by the net-like tissue of the whole pharynx in *Dendrodoris*, which is very similar to the radula bearing cushions of other members of the Doridoidea, and by the cuticular lining.

Valdés *et al.* (1996) misinterpreted the different parts of the proximal digestive system. They used the term pharyngeal bulb for a part which represents the oral tube and they described the pharynx (anterior to the salivary glands) as part of the oesophagus. Our histological results definitely show the homology of the anterior parts with the muscular bulb of other radular-bearing gastropods.

Usually the salivary glands have a uniform structure in Nudibranchia, with at least two different types of glandular cells, one of them producing acid mucopolysaccharides (unpublished results of senior author). *Dendrodoris nigra* has only one type which, according to the staining results, does not produce acid mucopolysaccharides. So, the structure of the salivary glands, as well as their histology, seems to be unique within the Nudibranchia.

The oesophagus of *Dendrodoris nigra* (and also *D. fumata*, unpublished results) is also unique in its structure, having virtually no lumen and by being composed exclusively of glandular cells. Similar glandular cells can be found in *Jorunna tomentosa* and *Hypselodoris tricolor*, but not in this quantity nor thickness. Furthermore, in these species a lumen is always present. The family Phyllidiidae is sometimes united with the Dendrodorididae as *Porostomata* (Valdés 1996, Thollessen, 1998), but the oesophagus of *Phyllidia flava* differs in having no cells producing acid mucopolysaccharids. The cells in *Phyllidia* are elongate and only sparse, light blue tiny grana can be identified in the cytoplasm. No ciliated cells or special vacuolated cells were found in the oesophagus of *Dendrodoris nigra*. The thick layers of muscles around nearly the whole length of the oesophagus also seem to be unique. *Dendrodoris*, a sponge-eater, has no radula and it is assumed that the uptake of some food may occur by external digestion of the sponge-tissue before the actual sucking in of nutritive fluid takes place (Young 1969, Rudman 1998). According to our histological results, it seems probable that both the oral glands and the oesophagus secrete digestive enzymes. This secretion is expelled by contraction of the circular and longitudinal muscles surrounding the oesophagus. How far this musculature aids in sucking in the dissolved fluid, is not yet clear.

In the literature on *Dendrodoris*, the term "caecum" is used for a sac-like enlargement of the intestine just at the point where it bends to take a posteriorly directed course (e.g. Brodie *et al.* 1997). Since a caecum in Doridoidea is assumed to represent the (much reduced) right digestive gland (Schmekel & Portmann 1982, Wägele 1989), this term should not imply homology but is used in the literature as a functional term. In *Dendrodoris nigra*, this part of the intestine is not differentiated histologically in any way. A real caecum (homologous to the right digestive gland) could not be detected. Since the stomach is not demarcated from the digestive gland in most parts, it was not even possible to identify that part of the digestive gland which could represent the right part in ontogenesis. For several Atlantic *Dendrodoris* species, Valdés *et al.* (1996) described a bulb-like structure in this area, which they termed "pyloric gland",

but their interpretation was not based on histological investigations. We cannot confirm any glandular function for this area in *Dendrodoris nigra* and would prefer to use the neutral term "pyloric bulb" for this structure.

The genital tract of *Dendrodoris nigra* is of the typical triaulic type (Schmekel 1970). According to observations on gonad structures in several doridoidean species (unpublished results of senior author), a gonad intermingled with the digestive gland and kidney is apparently unique. But further examination of other taxa has to follow before its value for phylogenetic analyses can be discussed. From the contents of the receptaculum seminis and bursa copulatrix, we can conclude that all animals have mated at least once, although only the male stage was fully developed in the smallest animal and no oocytes were present in the gonad of this specimen. The entrance of the vestibular gland into the oviduct, next to its transition into the vestibulum is quite a long way away from the openings of vaginal duct and vas deferens. This gives the impression that the function of the vestibular gland has more to do with egg laying than receipt of sperm. The tall border of microvilli in the glandular epithelium of the vestibular gland in larger specimens is also very unusual. According to Brodie & Klussmann-Kolb (1998) they house many bacteria. Up to now, only a few species of *Dendrodoris* were known to possess a vestibular gland (e.g. *D. elongata*, Baba 1994, *D. goani* Rao & Kumary 1973). In our smallest specimen the vestibular gland was still lying within the notal tissue which suggests it might not have been visible when performing a dissection. In the light of this result, previous studies on smaller and lesser known species of the Dendrodorididae should be re-investigated.

In all three specimens examined, no cuticular structures were present at the penis. Penial spines are thought to be typical of mature *Dendrodoris nigra* (Kay & Young 1969, Edmunds 1971, Brodie *et al.* 1997), although this conclusion usually rests on only a few specimens. The absence of spines was also mentioned for only one specimen of *Dendrodoris fumata* by Brodie *et al.* (1997). They interpreted this as an artefact. It needs to be clarified, by a comparative analysis of many specimens, whether there exists a high intraspecific variability. On the basis of this, it must also be questioned as to whether the great emphasis Valdés *et al.* (1996) placed on the penial armature of *Dendrodoris* species as species-specific, is reasonable. According to their descriptions and figures, the differences in the spine structure of some of the species they diagnose according to this character can also be interpreted as different stages in spine development.

Pericardial glands are present in the dorsal pericardial wall of *Dendrodoris nigra*, just as in some Doridoidea (including *Phyllidia*, unpublished results of senior author). Although these folds are called glands, their function as glands is not evident from the histological results.

There is no doubt that *Dendrodoris* belongs to the taxon Doridoidea since it shares all autapomorphies with this taxon (oesophagus without cuticular lining; triaulic genital system; blood gland next to, or on top of, cerebral ganglia; presence of gill glands; Wägele 1997). To clarify the phylogenetic relationship of the Dendrodorididae within the Doridoidea is more difficult, because an extensive analysis of the doridoidean families does not exist and many characters discussed here are not well known for other doridoideans. Usually

the Doridoidea are separated into two major groups, the Cryptobranchia and Phanerobranchia. This classification is partly based on the presence or absence of a gill cavity into which the gills can be retracted. Although Gosliner and Johnson (1994) consider the presence of a gill cavity as the apomorphic trait, it can also be observed in the phanerobranch family Onchidorididae. Considering the fact that protection of the gills might increase fitness, evolution of a gill cavity and the formation of muscles to retract the gills (or vice versa) could have occurred several times.

The possession of retractile gills, with a gill cavity and gill retractors, plus the general shape (oval, with notum larger than foot), and especially the tripinnate gills which are not observed in Onchidorididae support the hypothesis of *Dendrodoris* being a cryptobranch dorid. Nevertheless, the monophyly of the Cryptobranchia and Phanerobranchia still has to be clarified, and we need much more information on all other doridoidean families before the phylogenetic relationship of the Dendrodorididae can be solved.

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